Amendments to the Specification:

Please amend the specification as shown:

Please delete the paragraph entitled "CROSS REFERENCE TO RELATED APPLICATIONS" on page 1 and replace it with the following paragraph:

This application claims priority to U.S. Provisional Applications No. ~~60/xxxx~~ 60/513,246, filed October 22, 2003, No. 60/500,736, filed September 05, 2003, and No. 60/430,202, filed December 02, 2002, each of which is incorporated herein by reference in their entirety.

Please delete the paragraph entitled "TECHNICAL FIELD OF THE INVENTION" on lines 9-11 of page 1 and replace it with the following paragraph:

The present invention provides methods and tools for rapidly detecting microorganisms such as molds and fungi, and acid and thermophilic ~~Alicyclobacillus spp and Geobaillus spp.~~ *Alicyclobacillus* spp and *Geobacillus* spp in test samples, particularly food samples.

Please delete the paragraph on page 1, lines 21-25 and page 2, lines 1-10, and replace it with the following paragraph:

Like fungi, many bacteria are ~~resitant~~ resistant to processing conditions, and some are resistant even to high acid conditions in food and beverage products. Alicyclobacilli are Gram-positive, spore-forming, aerobic rods classified as thermoacidophiles capable of growing at high temperatures and low pH (1, 2, 3). These bacteria, formerly of the *Bacillus* genus, were assigned into the new genus *Alicyclobacillus* in 1992 (1). Sequence analysis of the 16s rRNA genes proved that three previously classified *Bacillus* thermoacidophiles (*B. acidocaldarius*, *B. acidoterrestris*, and *B. cycloheptanicus)* belong in a group that differs from other closely related Bacilli. Additionally, a key phenotypic variation was found in the membrane composition of these three species. The primary fatty acid component in the membrane was determined to be ω–alicyclic fatty acids, a type of lipid not found in other *Bacillus* species at the time. This

evidence initiated the establishment of the *Alicyclobacillus* genus of obligate acidothermophiles, containing *A. acidocaldarius*, *A. acidoterrestris*, and *A. cycloheptanicus,* within the *Bacillus* branch (1). More recently, *A. hesperidum* and *Alicyclobacillus* genomic species 1 and 2 (24, 25), *A. acidiphilus* (22), *A. herbarius* (23), *A. sendaiensis* (26), and *A. pomorum* (27) have been added as new species within the genus *Alicyclobacillus.*

Please delete the paragraphs on page 3, lines 30-30 and page 4, lines 1-28, and replace them with the following paragraphs:

The present invention provides methods and kits for detecting the presence of *Alicyclobacillus* spp. and a closely related thermophilic bacterium, *Geobacillus,* in samples, particularly food samples. In one embodiment the method comprises, collecting bacterial cells in the sample, extracting DNA from the cells, and assaying for the presence of these bacterium species using a PCR technique, preferably real-time PCR, and three signature oligonucleotides (2 primers and a probe) directed to a particular sequence in a target gene encoding either the 16S rRNA or squalene-hopene cyclase (*shc*). (See the conserved sequences extending from nucleotide position 334 through nucleotide position 485, and from nucleotide position 752 through nucleotide position 813 of the ~~shc~~ *shc* gene sequence of Alicyclobacillus shown in Figure 5. Also see the conserved sequences extending from nucleotide position 1327 through nucleotide position 1460 of the 16S rRNA gene sequence of Alicyclobacillus shown in Figure 1.) The presence of multiple *Alicyclobacillus* spp. and a closely related thermophilic bacterium *Geobacillus* can be achieved within 3-5 hours using the described sample preparation procedures, and proper combination of the three oligonucleotides as primer-and-probe set in the real-time PCR reaction.

The kits of the present invention comprise at least one forward primer and one reverse primer, with or without a probe for amplifying a sequence of at least 50 consecutive nucleotides within a conserved region of the three ~~Alicyclobacillus~~ *Alicyclobacillus* spp. shown in Figure 1 (sequences shown in alignment). Figures 2, 3 and 4, respectively, show the full coding sequences for the 16S rRNA genes ~~fromo~~ from the *Alicyclobacillus* strains deposited with the ATCC as 43030, 49025, and 49029. In certain embodiments, the oligonucleotides comprise the entire or a majority of the following sequences or their reverse complement sequences, as a set or

as combination crossing multiple sets, e.g. in certain cases the forward primer of one set can be combined with a reverse primer that is based on the forward primer of another set. Thus the following embodiments can be used in various primer, probe, or primer-probe combinations. Depending on the primers that are combined, the lower oligo may be used as a probe. The sequence of the lower oligo corresponds to the coding sequence of the target region of the gene, and is complementary to the reverse primer in each set. The reverse primers are shown as the reverse complement of the targeted region of the gene. The forward primers correspond to the coding sequence of the target region of the gene.

Please delete the paragraph on page 7, lines 2-11, and replace it with the following paragraphs:

The present invention also provides methods and kits for detecting the presence of yeast and mold contaminants in samples, particularly in food samples. In one aspect, the method comprises collecting particulate matter, preferably cells and cellular fragments, in the sample, extracting DNA from the particulate matter, and assaying for the presence of yeast DNA in the extracted DNA using a PCR technique using primers that amplify a select conserved region in 18s rDNA of representative yeast species, including *Zygosaccharomyces bailii* (Lindner) Guilliermond strain ATCC 36947 and the other yeast species shown Figure 7. (See conserved sequence extending from nucleotide 81 through nucleotide 225 of the sequence of Z. ~~bali~~ bailii.) Preferably, the method uses real-time PCR, and three signature oligonucleotides (2 primers and a probe) directed to a particular sequence in the gene encoding the yeast 18S rDNA.

Please delete the paragraph on page 9, lines 25-27 and replace it with the following paragraph:

Figure 1 shows polynucleotide sequence alignmnent of 16S rRNA gene fragments from three representative strains of *Alicyclobacillus,* specifically, *A. acidocaldarius* ATCC43030 **(SEQ ID NO: 74)**, *A. acidoterrestris* ATCC49025 **(SEQ ID NO: 75)**, and *A. cycloheptanicus* ATCC49029 **(SEQ ID NO: 76). The consensus and majority sequences are shown in SEQ ID NOS 77-78, respectively.**

Please delete the paragraph on page 9, line 28 and replace it with the following paragraph:

Figure 2 shows the 16S rRNA gene coding Sequence for *A. cycloheptanicus* ATCC49029 **(SEQ ID NO: 136)**

Please delete the paragraph on page 9, line 29 and replace it with the following paragraph:
Figure 3 shows the 16S rRNA gene coding Sequence for *A. acidoterrestris* ATCC49025 **(SEQ ID NO: 135)**

Please delete the paragraph on page 9, line 30 and replace it with the following paragraph:
Figure 4 shows the 16S rRNA gene coding Sequence for *A. acidocaldarius* ATCC43030 **(SEQ ID NO: 134)**

Please delete the paragraph on page 10, lines 1-2 and replace it with the following paragraph:
Figure 5: shows the Shc gene sequence alignments for *A. cycloheptanicus* ATCC49029 and *A. acidoterrestris* ATCC49025 **(SEQ ID NOS 79-82, respectively, in order of appearance). The majority sequence is shown in SEQ ID NO: 140.**

Please delete the paragraph on page 10, lines 3-4 and replace it with the following paragraph:
Figure 6: shows the Shc amino acid sequence alignments for *A. cycloheptanicus* ATCC49029 and *A. acidoterrestris* ATCC49025 **(SEQ ID NOS 83-89, respectively, in order of appearance). The majority sequence is shown in SEQ ID NO: 90.**

Please delete the paragraph on page 10, lines 5-6 and replace it with the following paragraph:
Figure 7 shows the alignment for the 18s rDNA gene coding Sequence for Zygosaccaromyces, Penecillium digitatum, and Byssochlamys fulva **(SEQ ID NOS 91-104, respectively, in order of appearance). The primers and probes are shown in SEQ ID NOS 105-110, respectively, in order of appearance.**

Please delete the paragraph on page 10, lines 7-8 and replace it with the following paragraph:
Figure 8 shows the 16S rRNA gene coding sequence alignments for several strains of for *A. cycloheptanicus* **(SEQ ID NOS 111-120, respectively, in order of appearance). The consenus and majority sequences are shown in SEQ ID NOS 121-123, respectively, in order of**

appearance.

Please delete the paragraph on page 10, line 16 and replace it with the following paragraph:
Figure 12 shows the 18s rDNA gene coding Sequence for Zygosaccaromyces **(SEQ ID NO: 137)**

Please delete the paragraph on page 10, line 17 and replace it with the following paragraph:
Figure 13 shows the 18s rDNA gene coding Sequence for Penecillium digitatum **(SEQ ID NO: 138)**

Please delete the paragraph on page 10, line 18 and replace it with the following paragraph:
Figure 14 shows the 18s rDNA gene coding Sequence for Byssochlamys fulva **(SEQ ID NO: 139)**

Please delete the paragraph on page 11, line 20 and replace it with the following paragraph:
Figure 27 shows the ~~resuls f~~ results of Real-time PCR sensitivity test

Please delete the paragraph on page 11, lines 8-10 and replace it with the following paragraph:
Figure 22 shows the Alignment[a] **(SEQ ID NOS 124-133, respectively, in order of appearance)** of 134 bp priming region flanked by CC16S-F (CGTAGTTCGGATTGCAGGC)**(SEQ ID NO: 66)**, CC16S-Probe (CGGAATTGCTAGTAATCGC)**(SEQ ID NO: 67)**, and CC16S-R (CACGAGAGTCGGCAACAC)[b]**(SEQ ID NO: 68)**.

Please delete the paragraph on page 15, line 9 and replace it with the following paragraph:

Lyse Cells using standard ~~techiniques~~ techniques

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Please delete the paragraph on page 18, lines 14-22 and replace it with the following paragraph:
We have cloned and sequenced the 18s rDNA gene fragments of representative molds of food industry concerns, *Byssochlamys fulva* Olliver et Smith, teleomorph ATCC 24474 and *Penicillium digitatum* Saccardo, anamorph ATCC10030. Coloning primer up:TGCATGGCCGTTCTTAGTTGG **(SEQ ID NO: 69)**.(Z.B. code 64-75) (B.F. 667-688) (P.D.

674-695) down: GTGTGTACAAAGGGCAGGG **(SEQ ID NO: 70)** (Z.B. 417-237) (B.F. 1011-1031) (P.D. 1029-1049). We then compared our sequences against other published 18S rDNA sequences from molds, yeasts, and common eukarytic foods. We have also compared other target sequences including h1, h2, 23S rDNA, spacer sequence between 18S and 23S rDNA gene. We have developed primer and probe sequences that can detect the presence of generally all mold without cross-reacting with foods, yeast or bacteria. The aligned sequences of the 18S rDNA sequences of these yeast species are shown in Figure 17. Figures 12, 13 and 14 show the full coding sequences for the genes corresponding to the alignments shown in Figure 17.

Please delete Table V on page 27 and replace it with the following Table:

TABLE V. Oligonucleotide data for *Alicyclobacillus* spp. CC16S probe and primers.

Name	Sequence	Length	T_m	G+C content
CC16S-F	CGTAGTTCGGATTGCAGGC **(SEQ ID NO: 71)**	19 bp	65.6°C	57.9%
CC16S-R	GTGTTGCCGACTCTCGTG **(SEQ ID NO: 72)**	18 bp	63.3°C	61.1%
CC16S-Probe	CGGAATTGCTAGTAATCGC **(SEQ ID NO: 73)**	19 bp	57.9°C	47.4%